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A bradykinin (BK)$_1$ receptor antagonist blocks capsaicin-induced ear inflammation in mice

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1 The effect of various peptide antagonists on capsaicin-induced (250 μg per ear) ear inflammation has been examined.

2 Co-administration of the substance P (SP) antagonist [D-Pro^2,D-Trp^{7,9}]SP at 100 and 300 μg per ear with capsaicin markedly attenuated oedema, whereas a vasopressin antagonist was ineffective.

3 Using the same scheme, the mixed BK$_2$ and BK$_1$ bradykinin (BK) antagonist NPC 567 (D-Arg[Hyp^3, D-Phe^7]BK) did not inhibit oedema at 100 μg per ear, but did inhibit at a higher dose (300 μg). The BK$_1$ antagonist [Leu^8,desArg^9]BK produced significant inhibition at both doses.

4 When BK was used to induce ear inflammation (30 μg per ear), the SP antagonist inhibited ear oedema. Both BK receptor subtype antagonists inhibited inflammation with the BK$_1$ being more potent than the BK$_2$ antagonist.

5 These results suggest that BK$_1$ along with BK$_2$ receptors are located on capsaicin-sensitive fibres, where they may modulate the degree of neurogenic inflammation.

Introduction

It is well known that capsaicin specifically stimulates primary sensory pain fibres to release substance P (SP) and other tachykinins (Nagy *et al.*, 1981; Jancso *et al.*, 1985; Hoover, 1987). The release of SP, an 11 amino acid neuropeptide, by capsaicin and other noxious stimuli may be involved in the generation of local inflammatory responses via an axon reflex (Lembeck & Holzer, 1979). There is further evidence that bradykinin (BK) will also release SP from capsaicin-sensitive nerves (Butler & Hammond, 1980; Hakanson *et al.*, 1987; Geppetti *et al.*, 1988). BK itself is a nonapeptide involved in many biological processes. It is released during tissue damage, causing increased vascular permeability, vasodilatation, and pain (Regoli & Barabe, 1980). Since there appear to be at least two different types of receptors involved in mediating BK effects in tissues (Regoli & Barabe, 1980; Bouthillier *et al.*, 1987), it was of interest to determine which receptor subtype, BK$_1$ or BK$_2$, is involved in the control of neurogenic inflammation.

In most tissues BK acts on BK$_2$ receptors which are sensitive to BK and kallidin (Lys-BK) much more than to any other kinin. As originally noted by Regoli & Barabe (1980), vascular smooth muscle develops responsiveness to the normally inactive metabolites, desArg^{9}BK, and desArg^{10}kallidin. This subset of BK receptors has been termed type 'BK$_1$'. Further studies have shown that enhancement of BK$_1$ receptor induction *in vitro* is produced by exposure to lipopolysaccharide *in vivo* (Bouthillier *et al.*, 1987). In these experiments, development of a BK$_1$ receptor response requires several hours to be detectable.

Capsaicin-induced neurogenic inflammation in mouse ears has a rapid onset (unpublished data). Thus, this model of inflammation presented a simple assay to test whether the BK receptor controlling SP release was of the stable BK$_2$ or the inducible BK$_1$ subtype.

Methods

Groups of 6–10 male albino mice, 25–30 g (Charles River, NY) were anaesthetized with isoflurane. Vehicle or capsaicin (25 mg ml^{-1}) or capsaicin plus peptide was applied by a pipette to the right ear, 5 μl to both the dorsal and ventral surface. Animals were anaesthetized 30 min later, and killed by cervical dislocation. Both the right (treated) and left (untreated) ear were removed by cutting horizontally across the indentation at the base of the ear. Weights were determined to the nearest tenth of a mg.

The percentage inhibition of oedema formation was calculated as follows: the weight of the untreated ear was subtracted from the treated ear in each group (vehicle, irritant, drug). The mean irritant oedema was obtained by subtracting the average vehicle control ear weight from the average capsaicin-treated ear weight. The % inhibition of oedema by peptides was calculated by subtracting the average vehicle oedema value from each ear weight in the drug group, and that mean was divided by the difference between the irritant and vehicle groups. The resultant value was subtracted from one and multiplied by 100.

Capsaicin and peptides were dissolved in ethanol. For BK-induced oedema, BK was prepared with captopril, each at concentrations of 3 mg ml^{-1} to achieve a dose of 30 μg per ear. All peptides and peptide antagonists were dissolved in dimethyl sulphoxide. Peptides and drugs for these studies came from the following sources: capsaicin (Fluka, N.Y.); BK, [Leu^8,desArg^9]BK, [D-Pro^2,D-Trp^{7,9}]SP, and [1-mercaptocyclohexyl]acetyl-Tyr[Me]Arg^8-vasopressin (Sigma, St. Louis, MO.). NPC 567 (Arg[Hyp^3,D-Phe^7]BK) was synthesized at NOVA.

Statistical analysis was performed by Student's *t* test for unpaired observations.

Results

Inhibition of capsaicin oedema

Depending on the type of receptor antagonist, quantitatively different degrees of inhibition of capsaicin-induced oedema (250 μg per ear) were produced (Figure 1). At a concentration of 100 μg per ear, the SP antagonist, [D-Pro^2,D-Trp^{7,9}]SP inhibited inflammation by 42%, which was not significantly altered by increasing the dose to 300 μg per ear (52%). For comparison, an arginine vasopressin (AVP) receptor antagonist had no effect at 100 μg per ear. We tested the effects of BK by using both a mixed BK$_1$ and BK$_2$ receptor antagonist, NPC 567, and a specific BK$_1$ receptor antagonist, [Leu^8, desArg^9]BK. At 100 μg per ear, NPC 567 did not produce any appreciable inhibition of oedema, but at 300 μg it did cause a significant, 34%, inhibition of inflammation. On the other

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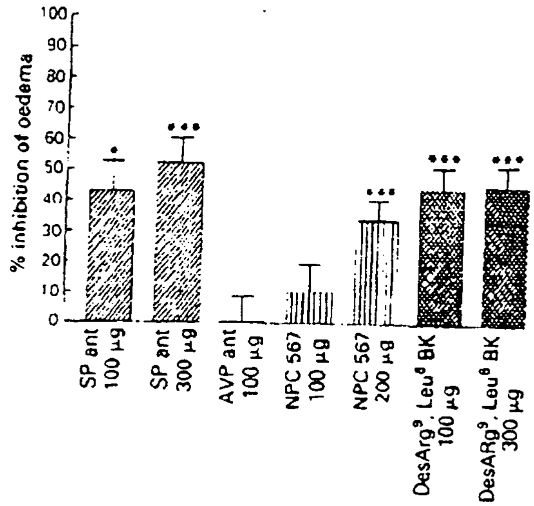

Figure 1 Inhibition of capsaicin oedema by various peptides. A dose of 250 µg capsaicin per ear produced a $36 \pm 1.0\%$ ($n = 6$) increase in ear weight. The substance P antagonist (SP ant) was [D-Pro^2,D-Trp^{7,9}] bradykinin (BK) and AVP ant = arginine vasopressin antagonist. Peptides were topically co-administered with capsaicin (250 µg) and oedema measured 30 min later. Values represent the mean of 20–30 mice, and vertical bars show s.e.mean. Statistically significant differences are represented as follows: $^*P < 0.05$, $^{***}P < 0.001$.

hand, the BK_1 antagonist caused 40% inhibition at 100 µg per ear, and non-significantly increased it to 45% at a dose of 300 µg.

BK-induced oedema

We investigated whether kinins can cause ear oedema, and the ability of various peptide antagonists to inhibit this inflammation (Figure 2). BK (30 µg) was a potent stimulant of inflammation when applied to the ear, the response was effectively inhibited (131%) by a single dose (300 µg) of NPC 567 applied to the ear. [Leu^8,desArg^9]BK produced a lower effect (27%) at 300 µg per ear (data not shown). The SP antagonist (100 µg) was as effective against BK-induced oedema (58%) as it was against capsaicin-induced oedema (Figure 1: 52%).

The dose-response relationship between oedema and the concentration of NPC 567 and [Leu^8,desArg^9]BK was investigated. In Figure 2, [Leu^8,desArg^9]BK is clearly shown to be a more potent inhibitor of oedema than NPC 567. In fact, the BK_1 antagonist reached a maximum of 100% inhibition at 30 µg per ear (Figure 2), then began to become less effective at higher concentrations (data not shown). Figure 2 demonstrates that the mixed BK_2 and BK_1 receptor antagonist, NPC 567, inhibited about 50% of the oedema produced, which is similar to the effect of [Leu^8,desArg^9]BK at low concentrations. At high concentrations of both antagonists there was a greater than 100% inhibition. This stems from the inhibitory effect on oedema produced by the DMSO vehicle alone. The peptide antagonist did not affect basal (non-vehicle) control ear weight. The linear transformation of the data indicated that the ED_{50} for NPC 567 was 16.2 µg per ear, whereas [Leu^8,desArg^9]BK had an ED_{50} of 2.0 µg per ear. Thus, based on these results, the existence of endogenous BK_1 receptors are implied.

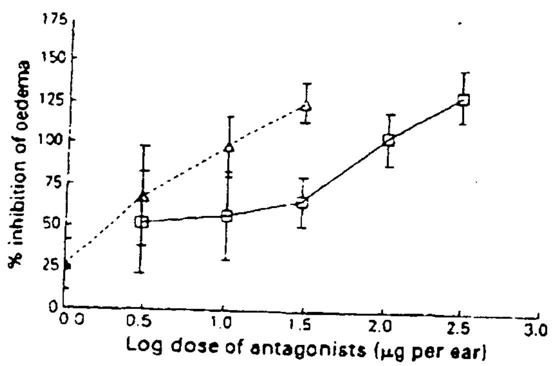

Figure 2 Dose-response curves for the inhibition of bradykinin-induced oedema by BK_1- and BK_2-receptor antagonists. BK itself produced $48 \pm 5.1\%$ ($n = 6$) oedema. The ED_{50} for NPC 567 (□) was 16.2 µg per ear and that for [Leu^8,desArg^9]BK (Δ) 2.0 µg per ear. Each point represents the mean of 10 mice and vertical lines show s.e.mean.

Discussion

Co-administration of a BK_1 receptor antagonist, [Leu^8,desArg^9]BK with capsaicin reduced neurogenic inflammation. Another peptide, D-Arg[Hyp^3,D-Phe^7]BK, which is a partial BK_2 receptor antagonist (Regoli *et al.*, 1986), was less effective at blocking this type of inflammation. That the SP antagonist, [D-Pro^2,D-Trp^{7,9}]SP, was effective at inhibiting capsaicin-induced oedema suggests that SP release by capsaicin may be the primary mediator of inflammation in this model.

Moreover BK-induced inflammation in the mouse ear was also strongly inhibited by the same SP antagonist. Others have shown that the intravenous injection of an SP antagonist reduces BK-induced vascular permeability (Shibata *et al.*, 1986). The effects of these peptide antagonists were specific. For example, a vasopressin receptor antagonist did not inhibit oedema formation induced by capsaicin. These results suggest that both BK_1 and BK_2 receptors are able to modify the capsaicin-induced release of SP, and thus influence the development of neurogenic inflammation.

BK_1 receptors have characteristically been shown not to be present in normal tissue, but synthesized upon tissue injury (Regoli & Barabe, 1980). This can also be seen when rabbits are treated intravenously with endotoxin where BK_1 receptor-mediated hypotension is seen 6 h after treatment (Bouthillier *et al.*, 1987). Capsaicin-induced inflammation, as measured by an increase in ear weight, is already maximal at 30 min post application (data not shown). This suggests that BK_1 receptors are normally present on sensory fibres in the mouse ear. BK and its kininase II metabolite desArg^{9}BK can then act at their respective receptors to amplify the inflammatory process further. In fact, BK has been shown to have a synergistic effect with SP on vascular permeability and the pain response in the mouse paw (Shibata *et al.*, 1986).

Thus, these data demonstrate the endogenous presence of BK_1 receptors on capsaicin-sensitive nerves without the *de novo* synthesis of these receptors with tissue injury. In the ear they appear to regulate the degree of neurogenic inflammation. Further studies are in progress to measure directly BK_1 receptors in ear tissue and elsewhere, by using radioligand binding techniques with desArg^{10}kallidin as the ligand.

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108: 887t **The study on vasopressin receptors in the cochlea.** Mori, Nozomu; Shugyo, Akio; Asai, Hideyo; Matsunaga, Toru (Med. Sch., Osaka Univ., Osaka, Japan). *Ear Res. Jpn.* **1987**, 18, 44–6 (Japan). To det. which receptor mediates the action of vasopressin on the endocochlear potential, the effect of V_1 antagonist and V_2 agonist on the endocochlear potential (EP) was examd. in the guinea pig. The V_1 antagonist failed to block the EP change induced by AVP, but the V_2 agonist produced reversible EP redn. like AVP. Evidently, the V_2 receptor mediates the action of vasopressin on the EP. N. Yago